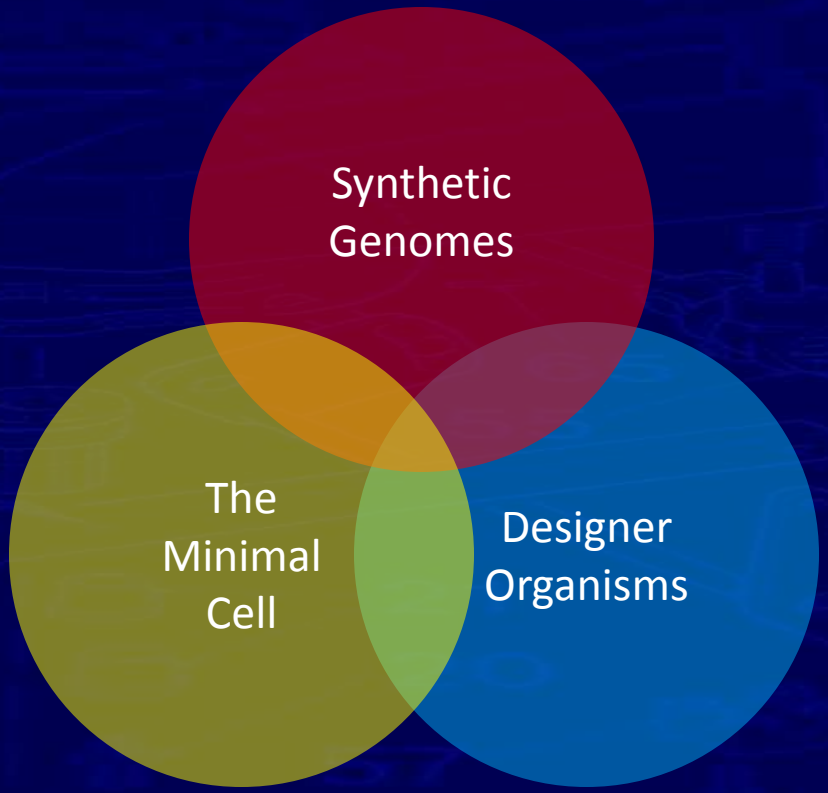
Synthetic Biological Systems

2. Synthetic Life and Genome Engineering

The construction of synthetic organisms

Synthesising biological life will be a 21st Century Grand Challenge



Fascinating big-ticket projects

Making the minimal cell

- Bottom-up approach to build from parts
- Top-down approach to reduce natural cells

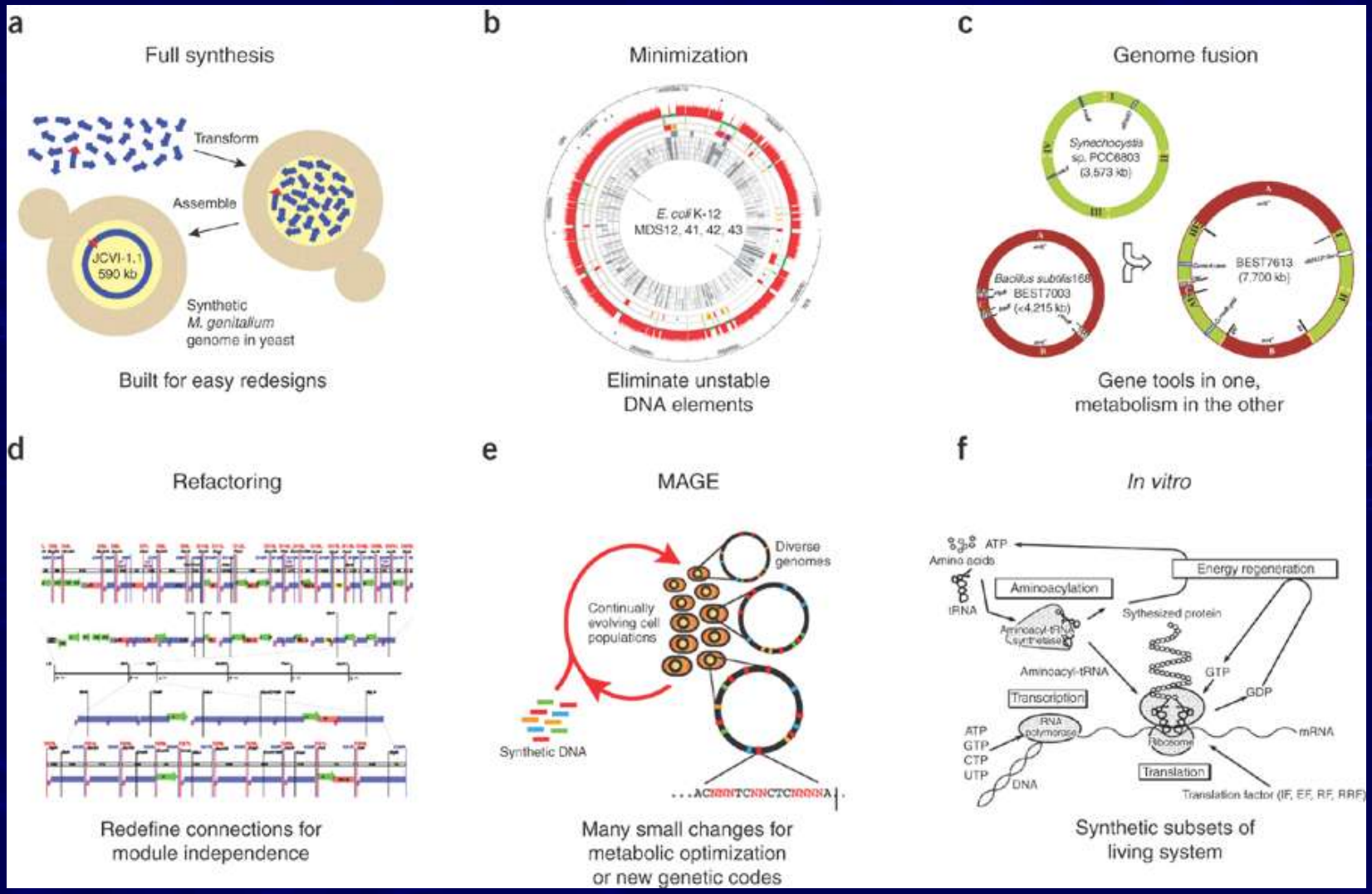
Building the first synthetic organism

- J. Craig Venter Institute

Re-factoring a genome

- Re-write the genetics of a cell to suit our needs
- “Genomic Engineering”

Genome Engineering



The first synthetic organism

The 1st synthetic organism – life made from a chemically synthesized genome

tRNA gene synthesized – Nobel Prize for Khorana

Phage/Virus genomes synthesized – synthesis of polio virus 2002

Viruses re-factored – separate each gene in a human-designed logical way

Next... Bacteria

A big two-part project by the J Craig Venter Institute

Part 1: Can a complete DNA genome be synthesized from chemicals	2008
Part 2: Can a cleaned DNA genome boot-up a cell	2007
Synthetic Organism = Parts 1 and Parts 2 combined	DOING

The first synthetic organism: (2) Booting-up

AIM: Genome A into Cell B → turn Cell B into Cell A

A: *M.mycodies* B: *M.capricolum* *different but compatible biology

C Lartigue *et al*. Science 2007

Comparable to nuclei-switch experiments in *In Vitro* Fertilisation

Genomes are fragile to handle in the lab – maintain in agarose plugs

How to get DNA into cell B? – incredibly inefficient, requires cell fusions (no cell wall)

What happens to genome of cell B? – doesn't have antibiotic resistance

Verify with sequencing, proteomics and phenotyping

Itaya *et al*.: fused complete genome of *Synechocystis* into *B.subtilis* - silencing

Holt Lab: build *H.influenzae* genome as BACs in *E.coli* - incompatible

The first synthetic organism: (1) Synthesis

AIM: Synthesize ~10^4 DNA 50-base oligomers and assemble into a complete error-free 582970 bp *M.genitalium* genome (watermarks)

1. Companies synthesise 101 pieces of 5 to 7 kb from overlapping oligos (e.g. Blue Heron and GeneArt)
2. 101 pieces recombined using *in vitro* enzymes to make 24 big pieces
3. 24 big pieces maintained in BACs in *E.coli* and recombined to make even bigger pieces
4. Big pieces all inserted into yeast and whole circular genome is made by recombination using native yeast genetics (using a YAC)
5. DNA sequencing used to check fidelity throughout process

DG Gibson *et al*. Science 2008

2010... Where's the synthetic organism? – restriction enzymes, methylation

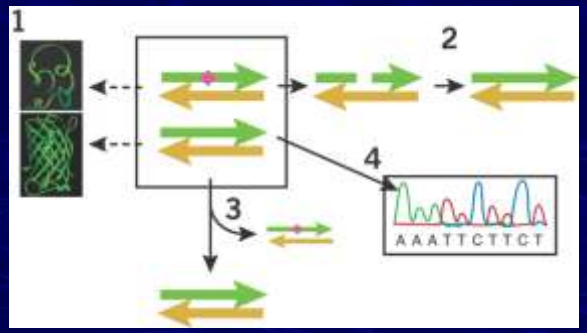
Genome Engineering Stages and Technologies

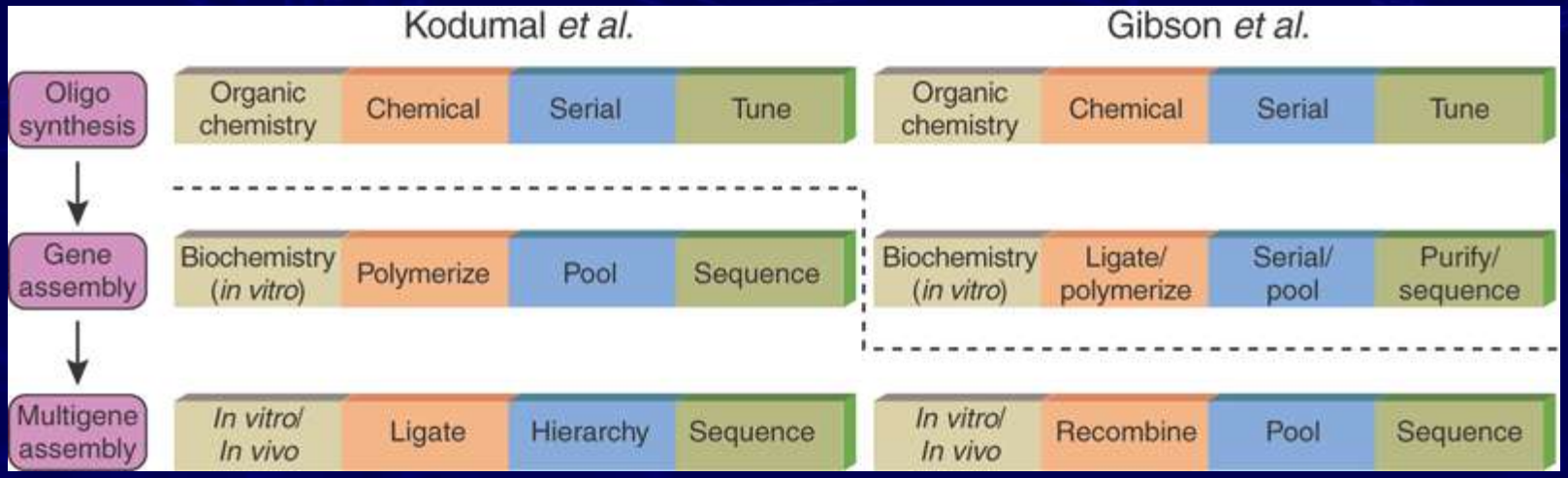
1. Synthesis — traditional oligo chemistry, on-chip polymerisation, cell factories

2. Joining — chemical, ligation, polymerisation, recombination

3. Assembly — serial, heirarchical, parallel, pooling

4. Error control — selection, tuning, repair, purification, sequencing





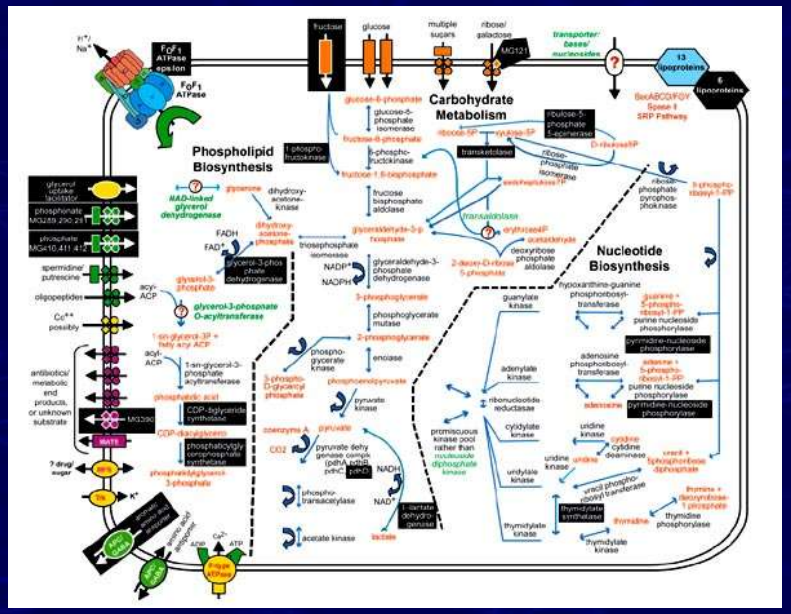
The minimal cell: top-down approach

Smallest natural genomes = 500 genes 500000 bps of DNA (e.g. *M.genitalium*)

But... not all genes are required for lab-based growth

How many essential genes?

1. Compare DNA throughout nature to identify essential genes
   Estimates: 50 to 380
2. Knock-out (delete) genes of small genomes to see what is needed
   Estimate: 430



Around a quarter of genes identified by these screens have unknown function

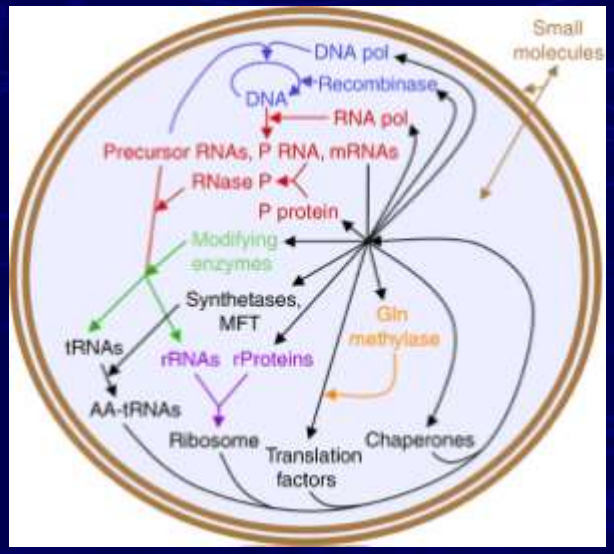
How do we really know that a gene is essential and not just playing many roles in a network?

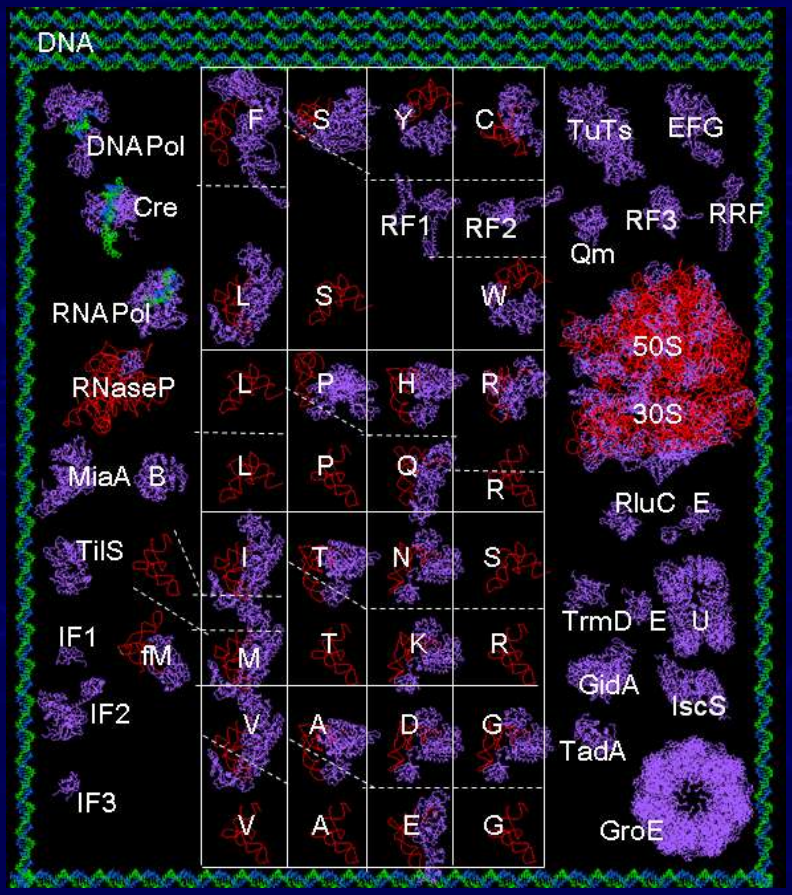
The minimal cell: bottom-up approach

"We know enough about a cell to identify the essential molecules and build our own from scratch" - hardcore synthetic biology

Biochemistry identifies the essential molecules that make cellular life

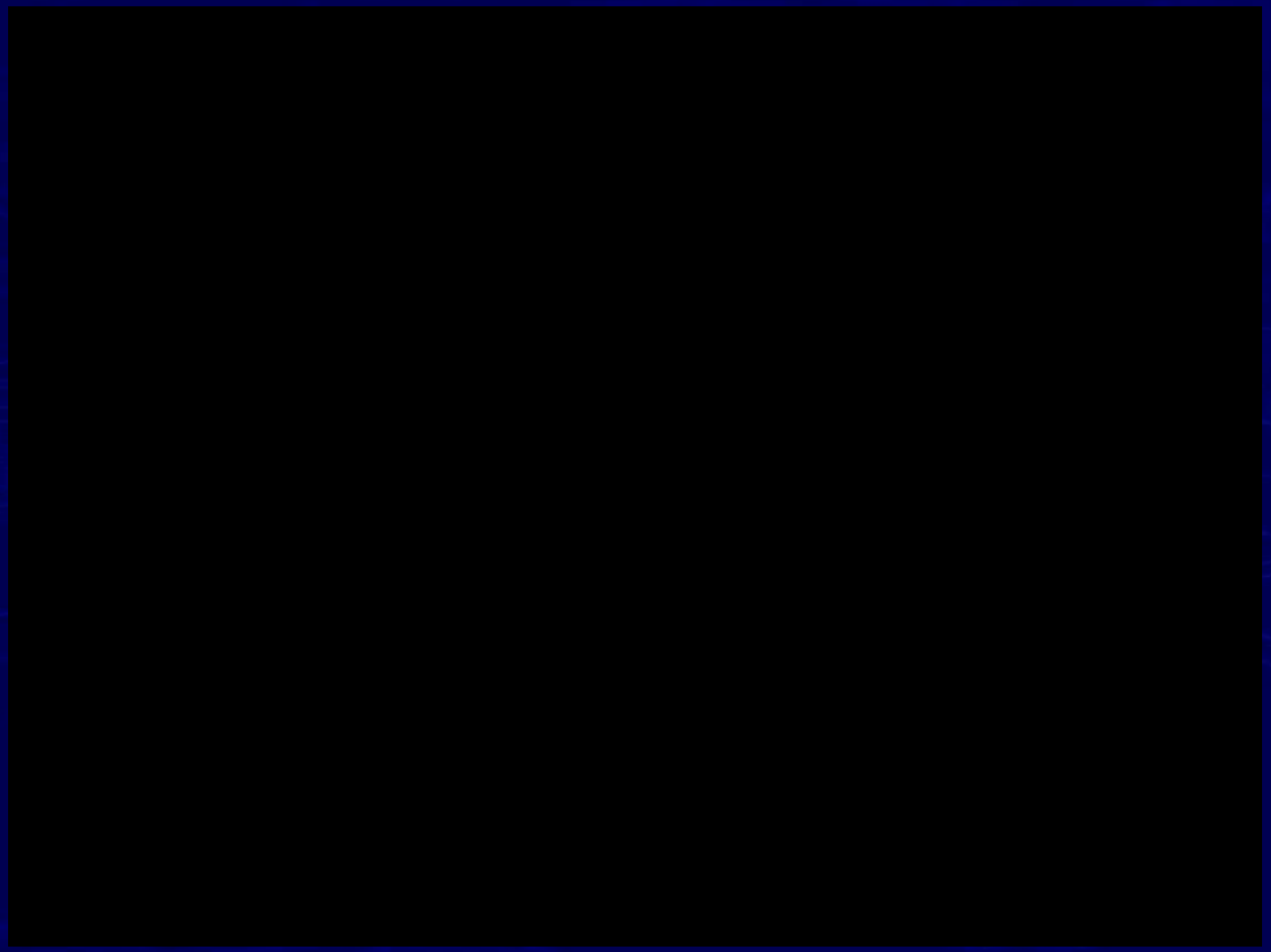
DNA → RNA → Protein





AC Forster & GM Church. Mol Sys Biol 2006

The minimal cell: bottom-up approach



The minimal cell: bottom-up approach

Just how many genes for a bottom-up minimal cell?

- Estimate: 151 genes = 38 RNAs + 113 proteins

Basic DNA replication 2	Chaperones 2	Ribosome 63
RNA transcription 1	RNA Processing 3	tRNA set 33
Translation Factors 11	AA-tRNA synthetases 21	tRNA modifiers 15

Would require all metabolites (eg. NTP) to be provided – no metabolism

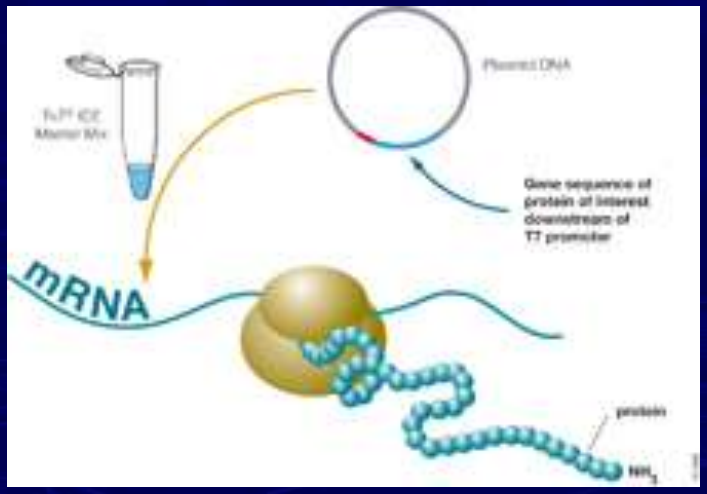
Would have no control over compartmentalisation – no membrane synthesis

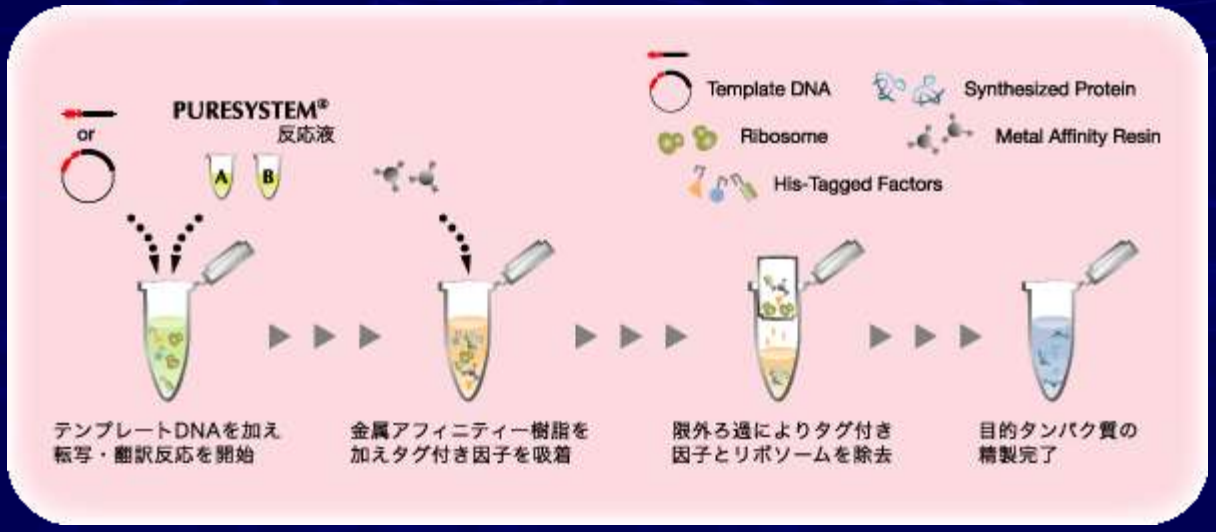
Really minimal cell – fragile *in vitro* system

Add metabolism, add lipid-synthesis for membranes, add proteins to control cell division, pores and transporters for sugar-import

Working minimal cell – capable of self-evolution

Existing *In Vitro* Transcription/Translation





Cell-free systems

Existing use:

- Protein synthesis for research and screening

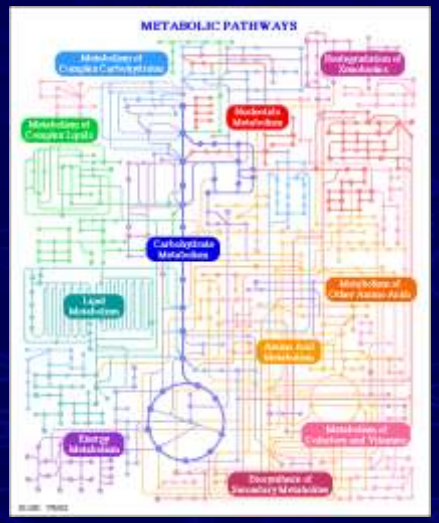
Planned use:

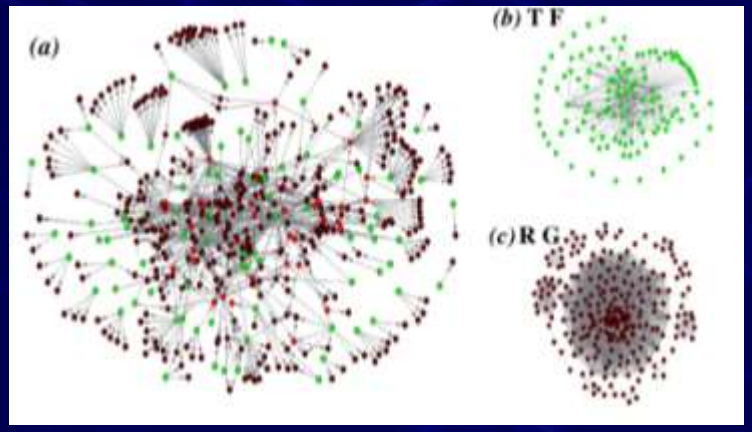
- Microfluidic systems - e.g. lab-on-a-chip
- Fast mutation and evolution of DNA

Modeling with Genome Engineering

A computational platform to design genomes : needs large-scale bottom-up models

1. Model the central core life functions – Replication, Transcription and Translation
2. Model metabolic networks and enzymes involved
3. Add regulation: a global transcriptional model
4. Improve the models with *in silico* directed evolution
5. Use the models to choose the organisation of genes on the genome
6. Try building versions and testing these



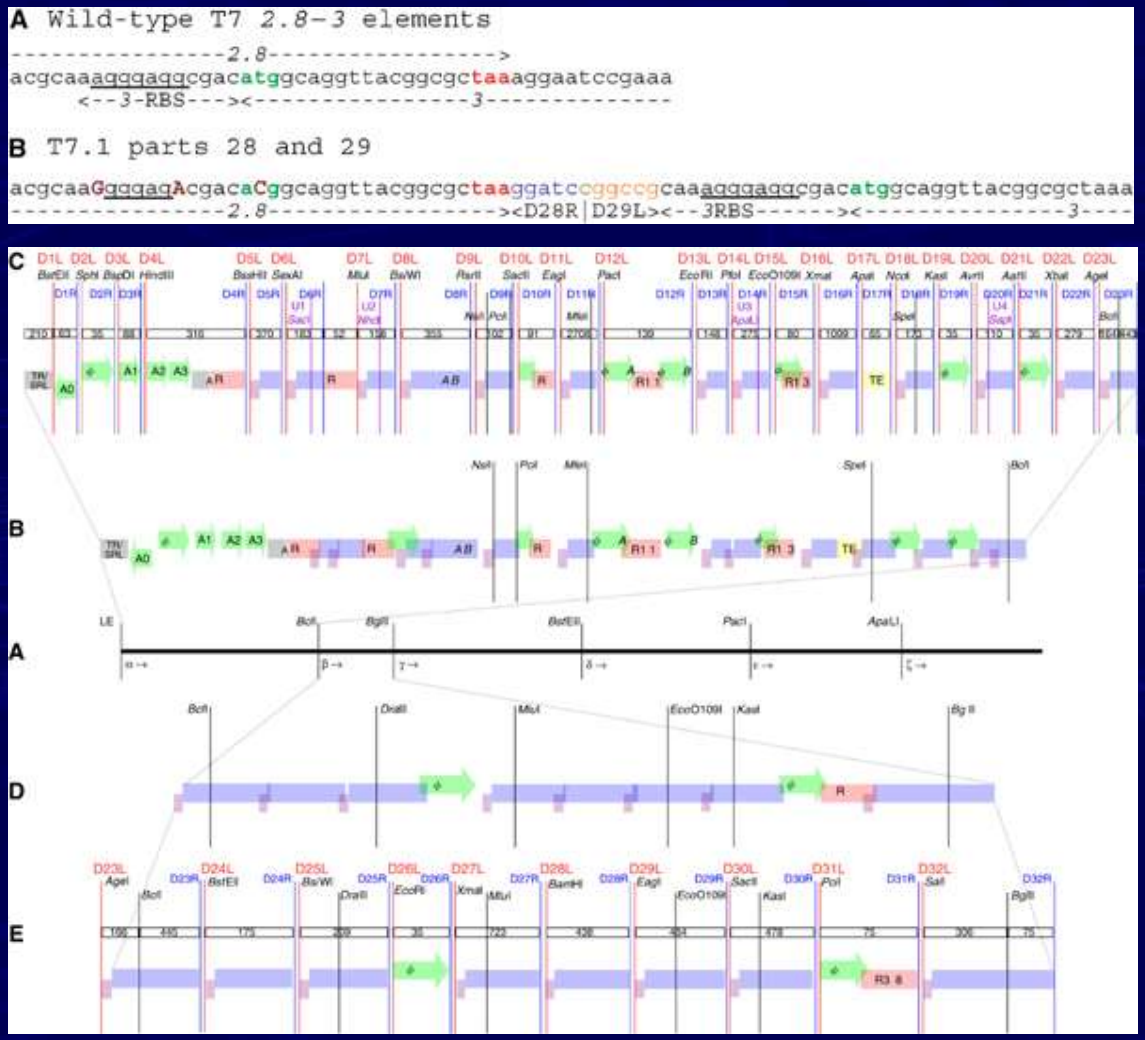


Re-factored genomes

Can we logically re-arrange a genome?

Add spacers, cut sites
Remove redundant DNA
Separate overlaps

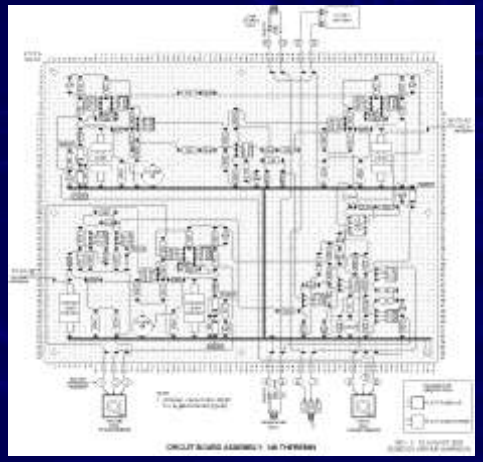
- M13 phage
- T7 phage
- Yeast chromosome (part of the synthetic yeast genome project)

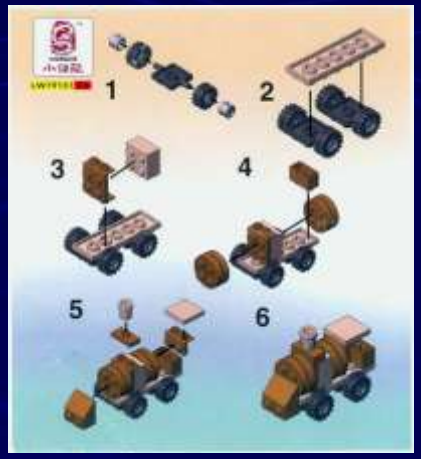


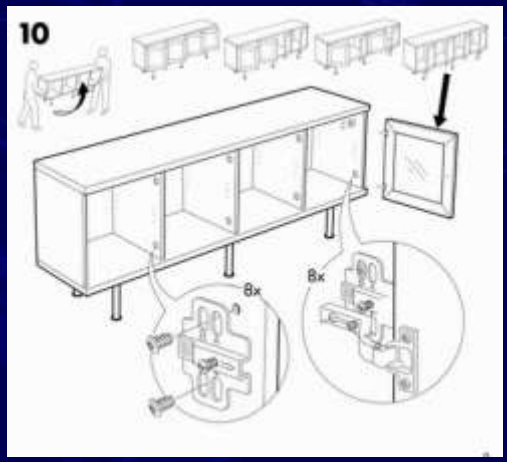
Why do genome engineering?

1. Bottom-up synthetic biology

- Adding genes and devices should be more predictable
- Creating a whole-cell model should be easier and allow better predictions of behaviour
- Provides a route to designing the chassis cell fit for a specific application
- Removal of unstable / recombination elements



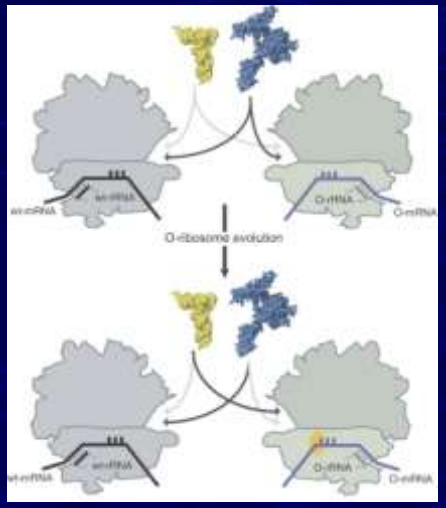


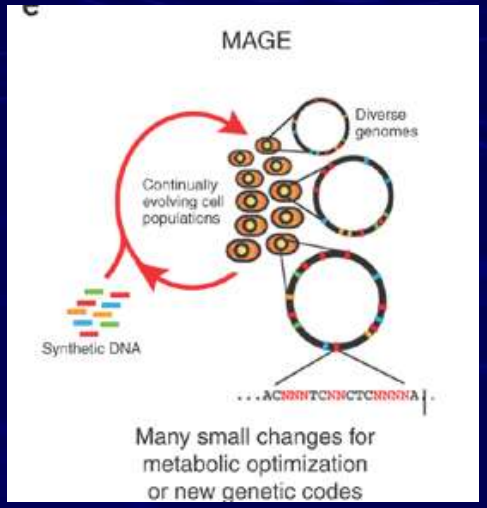


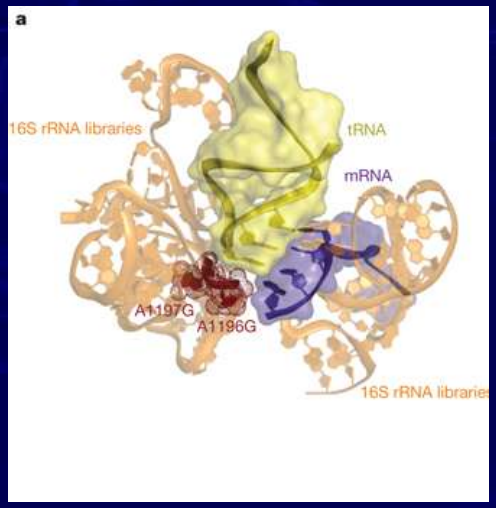
Why do genome engineering?

2. Provides for safer synthetic biology

- Cell can be designed to only survive in lab conditions
- Cell could be made " orthogonal" so that its biology doesn't interact with nature
- examples: change codon usage or change stereochemistry
- Better predictability from bottom-up design



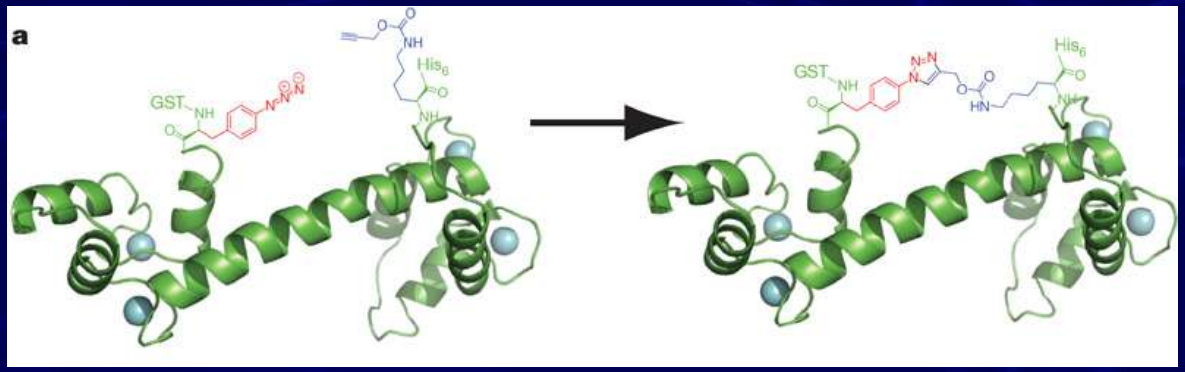




Why do genome engineering?

3. Custom synthesis of products

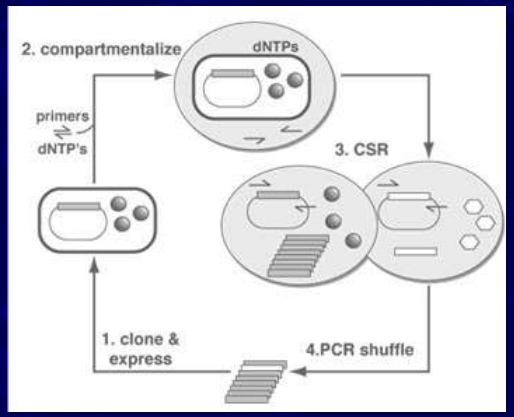
- Cells could be designed to produce non-natural proteins and sugars using synthetic building blocks
- Minimal cells would only use resources to make the desired products and so be more efficient
- Very cheap production of DNA could be engineered
- Synthesis of molecules that are toxic to produce in normal cells

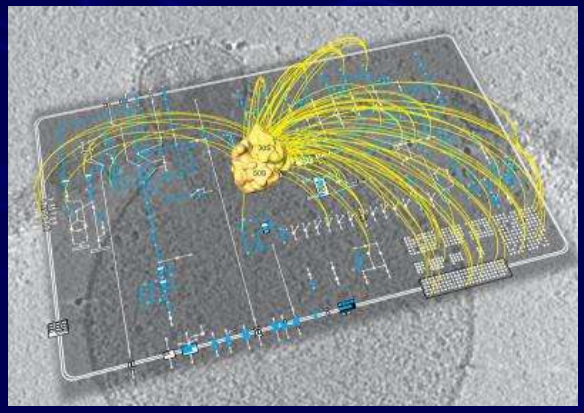


Why do genome engineering?

4. Other areas

- Minimal cell gives us a chance to study the origins of cellular life and potentially exobiology
- Fast evolution can be engineered to rapidly produce new enzymes
- Minimal cells would be easier to integrate into life-on-a-chip systems – e.g. a small screening device that sequences DNA, then synthesizes all the proteins from that DNA and compares their affinity to an antigen





Further Reading

Genome Engineering – PA Carr and GM Church

Nature Biotechnology, Vol. 27, No.12. (12 December 2009), pp. 1151-1162

Towards Synthesis of a Minimal Cell – AC Forster and GM Church

Molecular Systems Biology, Vol. 2 (22 August 2006)

Complete Chemical Synthesis, Assembly, and Cloning of a Mycoplasma genitalium Genome – DG Gibson et.al

Science, Vol. 319, No. 5867. (29 February 2008), pp. 1215-1220.

Artificial assembly of a minimal cell – G Murtas

Mol. BioSyst., Vol. 5, No. 11. (2009), pp. 1292-1297.

Towards the automated engineering of a synthetic genome – J Carrera, G Rodrigo and A Jaramillo

Mol. BioSyst., Vol. 5, No. 7. (July 2009), pp. 733-743.

The minimal cell: bottom-up approach